Baskar 09/846,808

=> d his 1

(FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 13:11:57 ON 28 OCT 2002)

L20 29 DUP REM L19 (65 DUPLICATES REMOVED)

=> d que l20

L1 3 SEA FILE=REGISTRY MSKRSNRKFVLWVMLILFTP.{0-}ALAMLSIGYYGGSIGIKFIL /SQSP
L2 2 SEA L1
L3 12740 SEA WALKER D?/AU
L4 8725 SEA YU X?/AU
L5 5163 SEA EHRLICHIA
L6 1355 SEA CHAFFEENSIS
L7 15148 SEA 28 (3A)(KD? OR KILODALTON?)
L8 37 SEA 28000 (3A) DALTON?
L9 4124465 SEA DNA OR RNA OR NUCLEIC OR RIBONUCLEIC OR DEOXYRIBONUCLEIC
L10 4 SEA AF230642
L11 3 SEA AF230643
L12 1949 SEA 23(3A)(KB OR KILOBASE#)
L13 2530 SEA P28
L15 62 SEA (L3 OR L4) AND (L5 OR L6) AND (L7 OR L8 OR L12 OR L13)
L17 102 SEA (L5 (3A) L6) AND (L7 OR L8 OR L12 OR L13)
L18 59 SEA L17 AND L9
L19 94 SEA L2 OR L10 OR L11 OR L15 OR L18
L20 29 DUP REM L19 (65 DUPLICATES REMOVED)

=> d ibib abs l20 1-29

L20 ANSWER 1 OF 29 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:609913 HCAPLUS
DOCUMENT NUMBER: 137:166520
TITLE: PCR primers and methods for detecting **Ehrlichia** canis and **Ehrlichia chaffeensis** in vertebrate and invertebrate hosts
INVENTOR(S): Stich, Roger William; Rikihisa, Yasuko
PATENT ASSIGNEE(S): The Ohio State University Research Foundation, USA
SOURCE: U.S., 36 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6432649	B1	20020813	US 2000-648520	20000825

AB Tools and methods for detecting the presence of E. canis and E. chaffeensis (the human granulocytic ehrlichiosis agent) in a sample obtained from an animal, such as human or dogs, are provided. The methods employ a polymerase chain reaction and primer sets that are based on the p30 gene of E. canis and the **p28** gene of E. chaffeensis. The present invention also relates to the p30 and the **p28** primer sets. Each p30 primer set comprises a first primer and the second primer, both of which are from 15 to 35 nucleotides in length. These primers are selected using criteria including annealing scores, identity of the primers to homologous E. chaffeensis sequences, and the availability of

Search completed by David Schreiber 308-4292

similarly optimal primers that are nested within the target template sequence. The methods are exemplified by detecting a 200bp-**DNA** fragment of E. canis p30 gene from the blood from dog carriers, or a 236bp-**DNA** fragment of E. chaffeensis **p28** gene from exptl. infected ticks of four species known to parasitize dogs. The p30-based assay is very sensitive than a previously reported nested 16S ribosomal **DNA** (rDNA)-based assay and only amplifies the 200-bp target amplicon from E. **chaffeensis** but not from **Ehrlichia** muris **DNA**. Optimized procedures for prepg. tissues from the infected hosts (dog carriers or infected ticks) and PCR conditions are described. The methods are useful for clin. diagnosis as well as exptl. investigations.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 2 OF 29 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 2

ACCESSION NUMBER: 2002:444530 HCAPLUS
DOCUMENT NUMBER: 137:29031
TITLE: Protein and DNA sequences of **Ehrlichia** canis homologous **28-kilodalton** immunodominant protein gene family and uses thereof
INVENTOR(S): **Walker, David H.; Yu, Xue-Jie;** McBride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: U.S., 42 pp., Cont.-in-part of U.S. Ser. No. 201,458. CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6403780	B1	20020611	US 1999-261358	19990303
US 6458942	B1	20021001	US 1998-201458	19981130
WO 2000032745	A2	20000608	WO 1999-US28075	19991124
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000019234	A5	20000619	AU 2000-19234	19991124
BR 9916141	A	20011204	BR 1999-16141	19991124
US 6392023	B1	20020521	US 2000-660587	20000912
US 2002115840	A1	20020822	US 2002-62624	20020131

PRIORITY APPLN. INFO.:
US 1998-201458 A2 19981130
US 1999-261358 A 19990303
WO 1999-US28075 W 19991124
US 2000-660279 A3 20000912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, ECa28-1, ECaSA2, and ECa28SA3, from a polymorphic multiple gene family of **Ehrlichia** canis. Further disclosed is a multigene locus encoding all five homologous **28-kDa** protein genes of **Ehrlichia** canis, and the five proteins are predicted to

have signal peptides resulting in mature proteins and had amino acid homol. ranging from 51 to 72%. Anal. of intergenic regions revealed hypothetical promoter regions for each gene, suggesting that these genes may be independently and differentially expressed. The invention further provides expression vectors comprising genes encoding the **28-kDa** immunoreactive proteins and capable of expressing the gened when the vectors are introduced into cells. The invention discloses that the recombinant **Ehrlichia** canis **28-kDa** proteins react with convalescent phase antiserum from an E. canis-infected dog.

REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 3 OF 29 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 2002:387621 HCAPLUS
DOCUMENT NUMBER: 136:381390
TITLE: Protein and DNA sequences of homologous **28-kilodalton** immunodominant protein genes of **Ehrlichia** canis and therapeutical uses
INVENTOR(S): **Walker, David H.; Yu, Xue-Jie;** McBride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: U.S., 49 pp., Cont.-in-part of U.S. Ser. No. 261,358. CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6392023	B1	20020521	US 2000-660587	20000912
US 6458942	B1	20021001	US 1998-201458	19981130
US 6403780	B1	20020611	US 1999-261358	19990303
WO 2002022782	A2	20020321	WO 2001-US28759	20010912
WO 2002022782	A3	20020530		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2001090926	A5	20020326	AU 2001-90926	20010912

PRIORITY APPLN. INFO.:

US 1998-201458	A2	19981130
US 1999-261358	A2	19990303
US 2000-660587	A	20000912
WO 2001-US28759	W	20010912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia** canis. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia** canis. The invention also provides expression vectors comprising genes encoding the **28-kDa** proteins which are capable of expressing the recombinant proteins when the vectors are introduced into a cell. The **Ehrlichia** canis

28-kDa proteins react with convalescent phase antiserum from an E. canis-infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 4 OF 29 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:220752 HCAPLUS
DOCUMENT NUMBER: 136:242995
TITLE: Homologous **28-kDa** immunodominant outer membrane protein genes of **Ehrlichia** canis and uses thereof for dog vaccine preparation to treat related infection
INVENTOR(S): **Walker, David H.; Yu, Xue-Jie;** Mcbride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 106 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002022782	A2	20020321	WO 2001-US28759	20010912
WO 2002022782	A3	20020530		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 6392023	B1	20020521	US 2000-660587	20000912
AU 2001090926	A5	20020326	AU 2001-90926	20010912

PRIORITY APPLN. INFO.:
US 2000-660587 A 20000912
US 1998-201458 A2 19981130
US 1999-261358 A2 19990303
WO 2001-US28759 W 20010912

AB The present invention is directed to the cloning, sequencing and expression of homologous immunoreactive **28-kDa** outer membrane protein genes, **p28**-1, -2, -3, -5, -6, -7, -9, from a polymorphic multiple gene family of **Ehrlichia** canis. Further disclosed is a multigene locus encoding all nine homologous **28-kDa** protein genes of **Ehrlichia** canis. Recombinant **Ehrlichia** canis **28-kDa** proteins react with convalescent phase antiserum from an E.canis-infected dog, and may be useful in the development of vaccines and serodiagnostics that are particularly effective for disease prevention and serodiagnosis. The invention also relates to methods and compns. directed toward the prevention of E. canis infection of dogs.

L20 ANSWER 5 OF 29 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:278689 BIOSIS
DOCUMENT NUMBER: PREV200200278689

Search completed by David Schreiber 308-4292

Baskar 09/846,808

TITLE: P43 antigen for the immunodiagnosis of canine ehrlichiosis and uses thereof.
AUTHOR(S): **Walker, David H. (1)**; McBride, Jere W.
CORPORATE SOURCE: (1) Galveston, TX USA
ASSIGNEE: Research Development Foundation
PATENT INFORMATION: US 6355777 March 12, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 12, 2002) Vol. 1256, No. 2, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB Canine monocytic ehrlichiosis, caused by Ehrilichia canis is a potentially fatal disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. In the invention described herein, a new immunoreactive E. canis surface protein gene of 1170-bp was cloned, which encodes a protein with a predicted molecular mass of 42.6 kilodaltons (P43). The P43 gene was not found in E. **chaffeensis** DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. **chaffeensis** by IFA. The P43 was located on the surface of E. canis by immunoelectron microscopy. Forty-two dogs exhibiting signs and/or hematologic abnormalities associated with canine ehrlichiosis were tested by IFA and by Western immunoblot. Among the 22 samples that were IFA positive for E. canis, 100% reacted with the rP43, 96% with the rP28, and 96% with the rP140. The specificity of the recombinant proteins compared to IFA was 96% for rP28, 88% for P43 and 63% for P140. Results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for the diagnosis of **Ehrlichia** canis infections.

L20 ANSWER 6 OF 29 MEDLINE
ACCESSION NUMBER: 2002372342 MEDLINE
DOCUMENT NUMBER: 22112924 PubMed ID: 12117987
TITLE: The omp-1 major outer membrane multigene family of **Ehrlichia chaffeensis** is differentially expressed in canine and tick hosts.
AUTHOR: Unver Ahmet; Rikihisa Yasuko; Stich Roger W; Ohashi Norio; Felek Suleyman
CORPORATE SOURCE: Department of Veterinary Biosciences, The Ohio State University, Columbus 43210-1093, USA.
CONTRACT NUMBER: R01AI40934 (NIAID)
R01AI47407 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (2002 Aug) 70 (8) 4701-4.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 20020716
Last Updated on STN: 20020906
Entered Medline: 20020904
AB Sixteen of 22 omp-1 paralogs encoding **28-kDa**-range immunodominant outer membrane proteins of **Ehrlichia chaffeensis** were transcribed in blood monocytes of dogs throughout a 56-day infection period. Only one paralog was transcribed by E. chaffeensis in three developmental stages of Amblyomma americanum ticks before or after E. chaffeensis transmission to naive dogs.

Search completed by David Schreiber 308-4292

L20 ANSWER 7 OF 29 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2002164185 MEDLINE
DOCUMENT NUMBER: 21893092 PubMed ID: 11895944
TITLE: Antigenic variation of **Ehrlichia chaffeensis** resulting from differential expression of the **28-kilodalton** protein gene family.
AUTHOR: Long S Wesley; Zhang Xiao-Feng; Qi Hai; Standaert Steven; **Walker David H; Yu Xue-Jie**
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, Texas 77555-0609, USA.
CONTRACT NUMBER: AI 45871 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (2002 Apr) 70 (4) 1824-31. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 20020317
Last Updated on STN: 20020412
Entered Medline: 20020411

AB The transcriptional activity and allele variation of the **28-kDa** outer membrane protein gene (**p28**) of **Ehrlichia chaffeensis** were analyzed to determine the mechanism of the antigenic variation of the **28-kDa** outer membrane proteins. Reverse transcriptase PCR amplification of mRNA indicated that 16 of the 22 members of the **p28** multigene family were transcribed. Amino acid sequence analysis indicated that the **p28**-19 protein was produced in vitro in the Arkansas strain. The **p28**-19 gene and its promoter region were sequenced and compared in 12 clinical isolates of E. **chaffeensis** to determine allele variation. The variation of the **p28**-19 gene among the isolates is limited to three types represented by strains Arkansas, 91HE17, and Sapulpa, respectively. These results indicate that the majority of the **p28** genes are active genes and that antigenic variation of the E. **chaffeensis 28-kDa** proteins may result from differential expression of the **p28** gene family members rather than gene conversion.

L20 ANSWER 8 OF 29 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2002116442 MEDLINE
DOCUMENT NUMBER: 21683633 PubMed ID: 11825969
TITLE: Detection of Ehrlichia canis in canine carrier blood and in individual experimentally infected ticks with a p30-based PCR assay.
AUTHOR: Stich Roger W; Rikihisa Yasuko; Ewing S A; Needham Glen R; Grover Debra L; Jittapalapong Sathaporn
CORPORATE SOURCE: Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, Ohio 43210-1092, USA.. stich.2@osu.edu
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (2002 Feb) 40 (2) 540-6. Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

Search completed by David Schreiber 308-4292

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020220
Last Updated on STN: 20020602
Entered Medline: 20020531

AB Detection of vector-borne pathogens is necessary for investigation of their association with vertebrate and invertebrate hosts. The ability to detect Ehrlichia spp. within individual experimentally infected ticks would be valuable for studies to evaluate the relative competence of different vector species and transmission scenarios. The purpose of this study was to develop a sensitive PCR assay based on oligonucleotide sequences from the unique Ehrlichia canis gene, p30, to facilitate studies that require monitoring this pathogen in canine and tick hosts during experimental transmission. Homologous sequences for **Ehrlichia chaffeensis p28** were compared to sequences of primers derived from a sequence conserved among E. canis isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. chaffeensis sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The p30-based assay was at least 100-fold more sensitive than a previously reported nested 16S ribosomal **DNA** (rDNA)-based assay and did not amplify the 200-bp target amplicon from E. chaffeensis, the human granulocytic ehrlichiosis agent, or Ehrlichia muris **DNA**. The assay was used to detect E. canis in canine carrier blood and in experimentally infected Rhipicephalus sanguineus ticks. Optimized procedures for preparing tissues from these hosts for PCR assay are described. Our results indicated that this p30-based PCR assay will be useful for experimental investigations, that it has potential as a routine test, and that this approach to PCR assay design may be applicable to other pathogens that occur at low levels in affected hosts.

L20 ANSWER 9 OF 29 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:816864 HCAPLUS
DOCUMENT NUMBER: 135:353851
TITLE: Identification of **Ehrlichia chaffeensis 28 kDa** outer membrane protein multigene family
INVENTOR(S): **Walker, David H.; Yu, Xue-Jie**
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 97 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001083699	A2	20011108	WO 2001-US13997	20010501
WO 2001083699	A3	20020404		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

Search completed by David Schreiber 308-4292

AU 2001059304	A5	20011112	AU 2001-59304		20010501
US 2002064531	A1	20020530	US 2001-846808		20010501
PRIORITY APPLN. INFO.:			US 2000-201035P	P	20000501
			WO 2001-US13997	W	20010501

AB The **28-kDa** outer membrane proteins (**P28**) of **Ehrlichia chaffeensis** are encoded by a multigene family consisting of 21 members located in a **23-kb DNA** fragment in the genome of E. **chaffeensis**. Fifteen of these proteins are claimed herein as novel sequences. The amino acid sequence identity of the various **P28** proteins was 20-83. Six of 10 tested **p28** genes were actively transcribed in cell culture grown E. **chaffeensis** RT-PCR also indicated that each of the **p28** genes was monocistronic. These results suggest that the **p28** genes are active genes and encode polymorphic forms of the **P28** proteins. The P28s were also divergent among different isolates of E. **chaffeensis**. The large repertoire of the **p28** genes in a single ehrlichial organism and antigenic diversity of the **P28** among the isolates of E.**chaffeensis** suggest that the P28s may be involved in immune avoidance.

L20 ANSWER 10 OF 29 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:816394 HCAPLUS
DOCUMENT NUMBER: 135:356748
TITLE: P43 antigen for the immunodiagnosis of canine ehrlichiosis and uses thereof
INVENTOR(S): **Walker, David H.**; McBride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001082862	A2	20011108	WO 2001-US13446	20010427
WO 2001082862	A3	20020404		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6355777	B1	20020312	US 2000-561322	20000428
AU 2001055702	A5	20011112	AU 2001-55702	20010427
PRIORITY APPLN. INFO.:			US 2000-561322 A	20000428
			WO 2001-US13446 W	20010427

AB Canine monocytic ehrlichiosis, caused by **Ehrlichia** canis is a potentially fatal disease of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. In the invention described herein, a new immunoreactive E. canis surface protein gene of 1170-bp was cloned, which encodes a protein with a predicted mol. mass of 42.6 kilodaltons (P43). The P43 gene was not found in E. **chaffeensis** DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. **chaffeensis**

by indirect fluorescent antibody (IFA). The P43 was located on the surface of E. canis by immunoelectron microscopy. Forty-two dogs exhibiting signs and/or hematol. abnormalities assocd. with canine ehrlichiosis were tested by IFA and by Western immunoblot. Among the 22 samples that were IFA pos. for E. canis, 100 reacted with the rP43, 96 with the rP28, and 96 with the rP140. The specificity of the recombinant proteins compared to IFA was 96 for rp28, 88 for P43 and 63 for P140. Results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for the diagnosis of **Ehrlichia** canis infections.

L20 ANSWER 11 OF 29 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2001574839 MEDLINE
DOCUMENT NUMBER: 21538942 PubMed ID: 11682500
TITLE: Identification of a **p28** gene in Ehrlichia ewingii: evaluation of gene for use as a target for a species-specific PCR diagnostic assay.
AUTHOR: Gusa A A; Buller R S; Storch G A; Huycke M M; Machado L J; Slater L N; Stockham S L; Massung R F
CORPORATE SOURCE: Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (2001 Nov) 39 (11) 3871-6.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20011030
Last Updated on STN: 20020314
Entered Medline: 20020313

AB PCR was used to amplify a 537-bp region of an Ehrlichia ewingii gene encoding a homologue of the **28-kDa** major antigenic protein (**P28**) of **Ehrlichia chaffeensis**. The E. ewingii **p28** gene homologue was amplified from **DNA** extracted from whole blood obtained from four humans and one canine with confirmed cases of infection. Sequencing of the PCR products (505 bp) revealed a partial gene with homology to outer membrane protein genes from Ehrlichia and Cowdria spp.: p30 of Ehrlichia canis (< or =71.3%), **p28** of E. chaffeensis (< or =68.3%), and map1 of Cowdria ruminantium (67.3%). The peptide sequence of the E. ewingii partial gene product was deduced (168 amino acids) and the antigenicity profile was analyzed, revealing a hydrophilic protein with < or =69.1% identity to **P28** of E. chaffeensis, < or =67.3% identity to P30 of E. canis, and < or =63.1% identity to MAP1 of C. ruminantium. Primers were selected from the E. ewingii **p28** sequence and used to develop a species-specific PCR diagnostic assay. The **p28** PCR assay amplified the expected 215-bp product from **DNA** that was extracted from EDTA-treated blood from each of the confirmed E. ewingii infections that were available. The assay did not produce PCR products with **DNA** extracted from E. chaffeensis-, E. canis-, or E. phagocytophila-infected samples, confirming the specificity of the **p28** assay for E. ewingii. The sensitivity of the E. ewingii-specific PCR assay was evaluated and determined to detect as few as 38 copies of the **p28** gene.

Search completed by David Schreiber 308-4292

L20 ANSWER 12 OF 29 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:240579 HCAPLUS
DOCUMENT NUMBER: 135:340042
TITLE: Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in **Ehrlichia** canis and E. **chaffeensis**
AUTHOR(S): Ohashi, Norio; Rikihisa, Yasuko; Unver, Ahmet
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210-1093, USA
SOURCE: Infection and Immunity (2001), 69(4), 2083-2091
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Ehrlichia** canis and E. **chaffeensis** are tick-borne obligatory intramonocytic ehrlichiae that cause febrile systemic illness in humans and dogs, resp. The current study analyzed the pleomorphic multigene family encoding approx. 30-kDa major outer membrane proteins (OMPs) of E. canis and E. chaffeensis. Upstream from secA and downstream of hypothetical transcriptional regulator, 22 paralogs of the omp gene family were found to be tandemly arranged except for one or two genes with opposite orientations in a 28- and a 27-kb locus in the E. canis and E. chaffeensis genomes, resp. Each locus consisted of three highly repetitive regions with four nonrepetitive intervening regions. E. canis, in addn., had a 6.9-kb locus which contained a repeat of three tandem paralogs in the 28-kb locus. These total 47 paralogous and orthologous genes encoded OMPs of approx. 30 to 35 kDa consisting of several hypervariable regions alternating with conserved regions. In the 5' -end half of the 27-kb locus or the 28-kb locus of each Ehrlichia species, 14 paralogs were linked by short intergenic spaces ranging from -8 bp (overlapped) to 27 bp, and 8 remaining paralogs in the 3' -end half were connected by longer intergenic spaces ranging from 213 to 632 bp. All 22 paralogs, five unknown genes, and secA in the omp cluster in E. canis were transcriptionally active in the monocyte culture, and the paralogs with short intergenic spaces were cotranscribed with their adjacent genes, including the resp. intergenic spaces at both the 5' and the 3' sides. Although omp genes are diverse, our results suggest that the gene organization of the clusters and the gene locus are conserved between two species of Ehrlichia to maintain a unique transcriptional mechanism for adaptation to environmental changes common to them.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 13 OF 29 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2001550448 MEDLINE
DOCUMENT NUMBER: 21480270 PubMed ID: 11596732
TITLE: Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in dogs from Thailand.
AUTHOR: Suksawat J; Xuejie Y; Hancock S I; Hegarty B C; Nilkumhang P; Breitschwerdt E B
CORPORATE SOURCE: Department of Veterinary Medicine, Faculty of Veterinary Medicine, Khon Kaen University, Thailand.
SOURCE: JOURNAL OF VETERINARY INTERNAL MEDICINE, (2001 Sep-Oct) 15 (5) 453-62.
Journal code: 8708660. ISSN: 0891-6640.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

Search completed by David Schreiber 308-4292

FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF082744; GENBANK-M83801; GENBANK-U26740
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011015
Last Updated on STN: 20020222
Entered Medline: 20020221

AB Forty-nine dogs from Thailand were evaluated for serologic evidence of exposure or polymerase chain reaction (PCR) evidence of infection with vectorborne pathogens, including Ehrlichia sp. (**Ehrlichia** canis, **Ehrlichia chaffeensis**, **Ehrlichia** equi, and **Ehrlichia** risticii), Bartonella vinsonii subsp. berkhoffi (Bvb), spotted fever group (SFG) rickettsiae (Rickettsia rickettsii), Typhus group (TG) rickettsiae (Rickettsia canada, Rickettsia prowazekii, and Rickettsia typhi), and Babesia sp. (Babesia canis and Babesia gibsonii). All study dogs had at least 1 of 3 entry criteria: fever, anemia, or thrombocytopenia. By immunofluorescence antibody (IFA) testing, seroreactivity was most prevalent to E chaffeensis (74%) and E canis (71%) antigens, followed by E equi (58%), Bvb (38%), E risticii (38%), R prowazekii (24%), B canis (20%), R rickettsii (12%), R canada (4%), and B gibsonii (4%) antigens. There was 100% concordance between E canis IFA and Western blot immunoassay (WI) for 35 of 35 samples; 2 samples were IFA and WI reactive only to E equi antigens. By PCR amplification, 10 dogs were found to be infected with E canis, 5 with Ehrlichia platys, and 3 with B canis. Sequencing of PCR products was undertaken to compare Ehrlichia strains from Thailand to strains originating from the United States. Partial **DNA** sequence analysis confirmed infection with E canis and E platys, with identical 16S rRNA sequence alignment to E canis (U26740) and to E platys (M83801), as reported in GenBank. Partial E canis **P28**.1 and **P28**.2 amino acid sequences from Thai dogs were divergent from analogous sequences derived from North American E canis (AF082744) strains, suggesting that the Thai dogs were infected with a geographically distinct strain of E canis compared to North American strains. The results of this study indicate that dogs in Thailand have substantial exposure to vectorborne diseases and that coinfection with these pathogens may be common.

L20 ANSWER 14 OF 29 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 2001131092 MEDLINE
DOCUMENT NUMBER: 20579049 PubMed ID: 11136790
TITLE: Immunodiagnosis of **Ehrlichia** canis infection with recombinant proteins.
AUTHOR: McBride J W; Corstvet R E; Breitschwerdt E B; **Walker D H**
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, Texas 77555, USA.
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (2001 Jan) 39 (1) 315-22. Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF252298
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301

AB **Ehrlichia** canis causes a potentially fatal rickettsial disease

of dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy leading to a favorable prognosis. We recently reported the cloning of two immunoreactive E. canis proteins, **P28** and P140, that were applicable for serodiagnosis of the disease. In the present study we cloned a new immunoreactive E. canis surface protein gene of 1,170 bp, which encodes a protein with a predicted molecular mass of 42.6 kDa (P43). The P43 gene was not detected in E. **chaffeensis** DNA by Southern blot, and antisera against recombinant P43 (rP43) did not react with E. **chaffeensis** as detected by indirect fluorescent antibody (IFA) assay. Forty-two dogs exhibiting signs and/or hematologic abnormalities associated with canine ehrlichiosis were tested by IFA assay and by recombinant Western immunoblot. Among the 22 samples that were IFA positive for E. canis, 100% reacted with rP43, 96% reacted with rP28, and 96% reacted with rP140. The specificity of the recombinant proteins compared to the IFAs was 96% for rP28, 88% for P43 and 63% for P140. The results of this study demonstrate that the rP43 and rP28 are sensitive and reliable serodiagnostic antigens for E. canis infections.

L20 ANSWER 15 OF 29 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:384370 HCAPLUS
DOCUMENT NUMBER: 133:27381
TITLE: Sequences of two novel homologous **28-kilodalton** immunodominant protein genes (ECa28-1 and ECa28SA3) of **Ehrlichia** canis and uses thereof
INVENTOR(S): **Walker, David H.; Yu, Xue-jie;** McBride, Jere W.
PATENT ASSIGNEE(S): Research Development Foundation, USA
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000032745	A2	20000608	WO 1999-US28075	19991124
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6458942	B1	20021001	US 1998-201458	19981130
US 6403780	B1	20020611	US 1999-261358	19990303
AU 2000019234	A5	20000619	AU 2000-19234	19991124
BR 9916141	A	20011204	BR 1999-16141	19991124

PRIORITY APPLN. INFO.:
US 1998-201458 A 19981130
US 1999-261358 A 19990303
WO 1999-US28075 W 19991124

AB The invention provides sequences of two novel homologous immunoreactive **28-kDa** protein genes, ECa28-1 and ECa28SA3, from a polymorphic multiple gene family of **Ehrlichia** canis. A complete sequence of another **28-kDa** protein gene, ECa28SA2, which was previously only partially sequenced, is also provided. Further

disclosed is a multigene locus (5.592-kb) encoding all five homologous **28-kDa** outer membrane protein genes (ECa28SA1, ECa28SA2, ECa28SA3, ECa28-1, and ECa28-2). Recombinant **Ehrlichia** canis **28-kDa** proteins react with convalescent phase antiserum from an E. canis-infected dog. The invention also relates to methods and compns. directed toward the prevention of E. canis infection of dogs.

L20 ANSWER 16 OF 29 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 2001013459 MEDLINE
DOCUMENT NUMBER: 20432107 PubMed ID: 10974556
TITLE: A conserved, transcriptionally active **p28** multigene locus of **Ehrlichia** canis.
AUTHOR: McBride J W; **Yu X J; Walker D H**
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX 77555-0609, USA.
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: GENE, (2000 Aug 22) 254 (1-2) 245-52.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF168788; GENBANK-AF168789
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001030

AB Antigenic diversity of **Ehrlichia chaffeensis** and **Ehrlichia** canis may involve independent or differential expression of the **P28** outer membrane proteins genes, enabling persistent infections of the natural hosts. In this study, we analyzed the transcriptional activity of a five gene locus in E. canis encoding homologous, but non-identical, **p28** genes. The **p28** multigene locus contained three previously identified complete gene sequences and one partial gene sequence. A new **p28** gene was identified and sequenced, and the complete sequence of a second partial **p28** gene was determined. The new **p28** gene joined two previously separate loci, forming the single **p28** multigene locus. The amino acid homology of the E. canis **P28** proteins ranged from 51 to 74%. The **nucleic** acid sequence of regions compared within the locus spanning four **p28** genes from two geographically distinct E. canis isolates was completely conserved. Analysis of the five **p28** genes demonstrated that all were transcriptionally active in in-vitro cultures of E. canis incubated at the vertebrate host (37 degrees C) and ambient tick temperatures (27 degrees C). Polycistronic copies of multiple **p28** genes were not detected by RT-PCR, but monocistronic **p28** mRNA transcripts were detected by Northern blotting from E. canis infected DH82 cells, indicating that the genes are transcribed as monocistronic messages.

L20 ANSWER 17 OF 29 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:3781 BIOSIS
DOCUMENT NUMBER: PREV200100003781
TITLE: Multiple **Ehrlichia** canis **p28** genes are transcriptionally active as monocistronic messages.
AUTHOR(S): McBride, J. W. (1); **Yu, X.-J. (1); Walker, D. H. (1)**
CORPORATE SOURCE: (1) Department of Pathology, University of Texas Medical

Branch, Galveston, TX USA
SOURCE: American Journal of Tropical Medicine and Hygiene, (March, 2000) Vol. 62, No. 3 Supplement, pp. 188. print.
Meeting Info.: 49th Annual Meeting of the American Society of Tropical Medicine and Hygiene Houston, Texas, USA October 29-November 02, 2000 American Society of Tropical Medicine and Hygiene
. ISSN: 0002-9637.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L20 ANSWER 18 OF 29 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:131804 HCAPLUS
DOCUMENT NUMBER: 133:191879
TITLE: Variability in the **28-kDa** surface antigen protein multigene locus of isolates of the emerging disease agent **Ehrlichia chaffeensis** suggests that it plays a role in immune evasion. [Erratum to document cited in CA131:285238]
AUTHOR(S): Reddy, Ganta Roman; Streck, Christopher P.
CORPORATE SOURCE: Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, 66056, USA
SOURCE: Molecular Cell Biology Research Communications (2000), 3(1), 66
CODEN: MCBCFS; ISSN: 1522-4724
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB On page 175, under Acknowledgments, the following should be added: "Part of the work reported in this article was supported by the USAID Grant LAG-1328-G-00-3030-00 at the University of Florida, Gainesville, FL.". (c) 2000 Academic Press.

L20 ANSWER 19 OF 29 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 2000267848 MEDLINE
DOCUMENT NUMBER: 20267848 PubMed ID: 10806351
TITLE: Characterization of the complete transcriptionally active **ehrlichia chaffeensis 28 kDa** outer membrane protein multigene family.
AUTHOR: **Yu X**; McBride J W; Zhang X; **Walker D H**
CORPORATE SOURCE: Department of Pathology, WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, TX 77555-0609, USA.. xuyu@utmb.edu
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: GENE, (2000 May 2) 248 (1-2) 59-68.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-**AF230642**; GENBANK-**AF230643**
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000714
Last Updated on STN: 20000714
Entered Medline: 20000706
AB The 28kDa outer membrane proteins (**P28**) of **Ehrlichia**

Search completed by David Schreiber 308-4292

chaffeensis are encoded by a multigene family. The purpose of this study was to determine all the **p28** gene sequences and their transcriptional activities. There were 21 members of the **p28** multigene family located in a 23kb **DNA** fragment in the genome of E. **chaffeensis**. The **p28** genes each contained 816-903 nucleotides with intergenic spaces of 10-605 nucleotides. All the genes were complete and were predicted to have a signal sequence. The molecular masses of the mature proteins were predicted to be 28-32kDa. The amino acid sequence identity of the **P28** proteins was 20-83%. Ten **p28** genes were investigated for transcriptional activity by using RT-PCR amplification of mRNA. Six of 10 tested **p28** genes were actively transcribed in cell-culture grown E. **chaffeensis**. RT-PCR also indicated that each of the **p28** genes was monocistronic. These results suggest that the **p28** genes are active genes and encode polymorphic forms of the **P28** proteins. The P28s were divergent among isolates of E. **chaffeensis** also. The large repertoire of the **p28** genes in a single ehrlichial organism and antigenic diversity of the **P28** among the isolates of E. **chaffeensis** suggest that P28s may be involved in immune avoidance.

L20 ANSWER 20 OF 29 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 1999335538 MEDLINE
DOCUMENT NUMBER: 99335538 PubMed ID: 10405403
TITLE: Comparison of **Ehrlichia chaffeensis** recombinant proteins for serologic diagnosis of human monocytotropic ehrlichiosis.
AUTHOR: **Yu X J**; Crocquet-Valdes P A; Cullman L C; Popov V L; **Walker D H**
CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77555-0609, USA.
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1999 Aug) 37 (8) 2568-75.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF117273
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990820
Last Updated on STN: 19990820
Entered Medline: 19990812

AB Diagnosis of human monocytotropic ehrlichiosis (HME) generally depends on serology that detects the antibody response to immunodominant proteins of **Ehrlichia chaffeensis**. Protein immunoblotting was used to evaluate the reaction of the antibodies in patients' sera with the recombinant E. **chaffeensis** 120- and **28-kDa** proteins as well as the 106- and the 37-kDa proteins. The cloning of the genes encoding the latter two proteins is described in this report. Immunoelectron microscopy demonstrated that the 106-kDa protein is located at the surfaces of ehrlichiae and on the intramorular fibrillar structures associated with E. **chaffeensis**. The 37-kDa protein is homologous to the iron-binding protein of gram-negative bacteria. Forty-two serum samples from patients who were suspected to have HME were tested by immunofluorescence (IFA) using E. **chaffeensis** antigen and by protein immunoblotting using recombinant E. **chaffeensis** proteins expressed in Escherichia coli. Thirty-two serum samples contained IFA

antibodies at a titer of 1:64 or greater. The correlation of IFA and recombinant protein immunoblotting was 100% for the 120-kDa protein, 41% for the **28-kDa** protein, 9.4% for the 106-kDa protein, and 0% for the 37-kDa protein. None of the recombinant antigens yielded false-positive results. All the sera reactive with the recombinant **28-** or the 106-**kDa** proteins also reacted with the recombinant 120-kDa protein.

L20 ANSWER 21 OF 29 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 1999175287 MEDLINE
DOCUMENT NUMBER: 99175287 PubMed ID: 10074538
TITLE: Genetic diversity of the **28-kilodalton** outer membrane protein gene in human isolates of **Ehrlichia chaffeensis.**
AUTHOR: **Yu X J**; McBride J W; **Walker D H**
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, The University of Texas Medical Branch at Galveston, Galveston, Texas 77555-0609, USA.
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1999 Apr) 37 (4) 1137-43.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF068234; GENBANK-AF068252; GENBANK-AF068253; GENBANK-AF068254; GENBANK-AF068255; GENBANK-AF068256; GENBANK-AF068257; GENBANK-AF068258; GENBANK-AF068259; GENBANK-AF068260; GENBANK-AF068261; GENBANK-AF068262; GENBANK-AF068263; GENBANK-AF077732; GENBANK-AF077733; GENBANK-AF077734; GENBANK-AF077735; +
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990504
Last Updated on STN: 19990504
Entered Medline: 19990419

AB The **Ehrlichia chaffeensis 28-kDa** outer membrane protein (**p28**) gene was sequenced completely by genomic walking with adapter PCR. The **DNA** sequence of the **p28** gene was nearly identical to the previously reported sequence (N. Ohashi, N. Zhi, Y. Zhang, and Y. Rikihisa, Infect. Immun. 66:132-139, 1998), but analysis of a further 75 bp on the 5' end of the gene revealed **DNA** that encoded a 25-amino-acid signal sequence. The leader sequence was removed from the N terminus of a 30-kDa precursor to generate the mature **p28** protein. A monoclonal antibody (MAb), 1A9, recognizing four outer membrane proteins of E. **chaffeensis** (Arkansas strain) including the 25-, 26-, 27-, and 29-kDa proteins (X.-J. Yu, P. Brouqui, J. S. Dumler, and D. Raoult, J. Clin. Microbiol. 31:3284-3288, 1993) reacted with the recombinant **p28** protein. This result indicated that the four proteins recognized by MAb 1A9 were encoded by the multiple genes of the **28-kDa** protein family. **DNA** sequence alignment analysis revealed divergence of **p28** among all five human isolates of E. **chaffeensis**. The E. **chaffeensis** strains could be divided into three genetic groups on the basis of the **p28** gene. The first group consisted of the Sapulpa and St. Vincent strains. They had predicted amino acid sequences identical to each other. The second group contained strain 91HE17 and strain Jax, which only showed 0.4% divergence from each other. The third group contained the Arkansas strain only. The amino acid

sequences of **p28** differed by 11% between the first two groups, by 13.3% between the first and third groups, and by 13.1% between the second and third groups. The presence of antigenic variants of **p28** among the strains of E. **chaffeensis** and the presence of multiple copies of heterogeneous genes suggest a possible mechanism by which E. **chaffeensis** might evade the host immune defenses. Whether or not immunization with the **p28** of one strain of E. **chaffeensis** would confer cross-protection against other strains needs to be investigated.

L20 ANSWER 22 OF 29 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 1999242757 MEDLINE
DOCUMENT NUMBER: 99242757 PubMed ID: 10225842
TITLE: Molecular cloning of the gene for a conserved major immunoreactive **28-kilodalton** protein of **Ehrlichia** canis: a potential serodiagnostic antigen.
AUTHOR: McBride J W; **Yu X j**; **Walker D H**
CORPORATE SOURCE: Department of Pathology and WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, Galveston, Texas 77555-0609, USA.
SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1999 May) 6 (3) 392-9.
Journal code: 9421292. ISSN: 1071-412X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF082744; GENBANK-AF082745; GENBANK-AF082746; GENBANK-AF082747; GENBANK-AF082748; GENBANK-AF082749; GENBANK-AF082750
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 19990925
Entered Medline: 19990916

AB A gene encoding a **28-kDa** protein of **Ehrlichia** canis was cloned, sequenced, and expressed, and a comparative molecular analysis with homologous genes of E. canis, Cowdria ruminantium, and **Ehrlichia chaffeensis** was performed. The complete gene has an 834-bp open reading frame encoding a protein of 278 amino acids with a predicted molecular mass of 30.5 kDa. An N-terminal signal sequence was identified, suggesting that the protein undergoes posttranslational modification to a mature 27.7-kDa protein (**P28**). The E. canis **p28** gene has significant **nucleic** acid and amino acid sequence homologies with the E. **chaffeensis** outer membrane protein-1 (omp-1) gene family, with the Cowdria ruminantium map-1 gene, and with other E. canis **28-kDa**-protein genes. Southern blotting revealed the presence of at least two additional homologous **p28** gene copies in the E. canis genome, confirming that **p28** is a member of a polymorphic multiple-gene family. Amino acid sequence analysis revealed that E. canis **P28** has four variable regions, and it shares similar surface-exposed regions, antigenicity, and T-cell motifs with E. **chaffeensis P28**. The **p28** genes from seven different E. canis isolates were identical, indicating that the gene for this major immunoreactive protein is highly conserved. In addition, reactivity of sera from clinical cases of canine ehrlichiosis with the recombinant **P28** demonstrated that the recombinant protein may be a reliable serodiagnostic antigen.

Search completed by David Schreiber 308-4292

Baskar 09/846,808

L20 ANSWER 23 OF 29 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:515994 HCAPLUS
DOCUMENT NUMBER: 131:285238
TITLE: Variability in the **28-kDa** Surface Antigen Protein Multigene Locus of Isolates of the Emerging Disease Agent **Ehrlichia chaffeensis** Suggests That It Plays a Role in Immune Evasion
AUTHOR(S): Reddy, Ganta Roman; Streck, Christopher P.
CORPORATE SOURCE: Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, 66506, USA
SOURCE: Molecular Cell Biology Research Communication (1999), 1(3), 167-175
CODEN: MCBCFS; ISSN: 1522-4724
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Infections caused by rickettsial pathogens persist in vertebrate hosts for long periods of time, despite the active host immune response. The authors recently described the multigene locus encoding **28 kDa** surface antigen proteins (**28 kDa** SAPs) for two closely related rickettsials, **Ehrlichia chaffeensis** and **Ehrlichia** canis, that share extensive structural homol. to antigenic variant surface protein genes of Neisseria and Borrelia species. In this study, the authors describe motifs for recombinase binding sites and a high frequency of repeat elements in the cloned **28 kDa** SAP genes. The locus for two newly established E. chaffeensis isolates also was characterized, and immunol. specificity of the **28 kDa** SAPs was investigated. The study identified variant forms of the **28 kDa** SAPs and extensive restriction fragment length polymorphisms among isolates. The mol. data suggest that the locus is influenced by short-term evolutionary changes such as genetic recombinations leading to the generation of antigenic variants. Antigenic variation could contribute to the mechanism of immune evasion and the emergence of new diseases. (c) 1999 Academic Press.
REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 24 OF 29 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 1998371112 MEDLINE
DOCUMENT NUMBER: 98371112 PubMed ID: 9705412
TITLE: Cloning and characterization of multigenes encoding the immunodominant 30-kilodalton major outer membrane proteins of Ehrlichia canis and application of the recombinant protein for serodiagnosis.
AUTHOR: Ohashi N; Unver A; Zhi N; Rikihisa Y
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210-1093, USA.
CONTRACT NUMBER: RO1 AI33123 (NIAID)
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1998 Sep) 36 (9) 2671-80.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF078553; GENBANK-AF078554; GENBANK-AF078555

Search completed by David Schreiber 308-4292

Baskar 09/846,808

ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980925
Last Updated on STN: 20000303
Entered Medline: 19980916

AB A 30-kDa major outer membrane protein of Ehrlichia canis, the agent of canine ehrlichiosis, is the major antigen recognized by both naturally and experimentally infected dog sera. The protein cross-reacts with a serum against a recombinant **28-kDa** protein (rP28), one of the outer membrane proteins of a gene (omp-1) family of **Ehrlichia chaffeensis**. Two **DNA** fragments of E. canis were amplified by PCR with two primer pairs based on the sequences of E. chaffeensis omp-1 genes, cloned, and sequenced. Each fragment contained a partial 30-kDa protein gene of E. canis. Genomic Southern blot analysis with the partial gene probes revealed the presence of multiple copies of these genes in the E. canis genome. Three copies of the entire gene (p30, p30-1, and p30a) were cloned and sequenced from the E. canis genomic **DNA**. The open reading frames of the two copies (p30 and p30-1) were tandemly arranged with an intergenic space. The three copies were similar but not identical and contained a semivariable region and three hypervariable regions in the protein molecules. The following genes homologous to three E. canis 30-kDa protein genes and the E. chaffeensis omp-1 family were identified in the closely related rickettsiae: wsp from Wolbachia sp. , p44 from the agent of human granulocytic ehrlichiosis, msp-2 and msp-4 from Anaplasma marginale, and map-1 from Cowdria ruminantium. Phylogenetic analysis among the three E. canis 30-kDa proteins and the major surface proteins of the rickettsiae revealed that these proteins are divided into four clusters and the two E. canis 30-kDa proteins are closely related but that the third 30-kDa protein is not. The p30 gene was expressed as a fusion protein, and the antibody to the recombinant protein (rP30) was raised in a mouse. The antibody reacted with rP30 and a 30-kDa protein of purified E. canis. Twenty-nine indirect fluorescent antibody (IFA)-positive dog plasma specimens strongly recognized the rP30 of E. canis. To evaluate whether the rP30 is a suitable antigen for serodiagnosis of canine ehrlichiosis, the immunoreactions between rP30 and the whole purified E. canis antigen were compared in the dot immunoblot assay. Dot reactions of both antigens with IFA-positive dog plasma specimens were clearly distinguishable by the naked eye from those with IFA-negative plasma specimens. By densitometry with a total of 42 IFA-positive and -negative plasma specimens, both antigens produced results similar in sensitivity and specificity. These findings suggest that the rP30 antigen provides a simple, consistent, and rapid serodiagnosis for canine ehrlichiosis. Cloning of multigenes encoding the 30-kDa major outer membrane proteins of E. canis will greatly facilitate understanding pathogenesis and immunologic study of canine ehrlichosis and provide a useful tool for phylogenetic analysis.

L20 ANSWER 25 OF 29 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 1998321180 MEDLINE
DOCUMENT NUMBER: 98321180 PubMed ID: 9647746
TITLE: Molecular characterization of a **28 kDa** surface antigen gene family of the tribe Ehrlichiae.
AUTHOR: Reddy G R; Sulsona C R; Barbet A F; Mahan S M; Burridge M J; Alleman A R
CORPORATE SOURCE: Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville 32610, USA.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Jun 29) 247 (3) 636-43.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States

Search completed by David Schreiber 308-4292

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF062761; GENBANK-AF062762
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980817
Last Updated on STN: 19980817
Entered Medline: 19980731

AB Antisera against different Ehrlichiae recognize an immunodominant, cross-reacting approximately **28 kDa** surface antigen defined as the MAP1 in Cowdria ruminantium. These antigens are considered valuable in developing serodiagnostic tests and recombinant vaccines for Ehrlichiae infections. To evaluate the relationship in three closely related Ehrlichiae, **Ehrlichia chaffeensis**, **Ehrlichia** canis, and C. ruminantium, the structure of the **28 kDa** antigen genes was analyzed. We describe the cloning and characterization of **DNA** encoding genes homologous to MAP1 from E. chaffeensis and E. canis. The cloned segment of E. chaffeensis contains one expressed and four transcriptionally silent tandemly arranged, nonidentical genes; the E. canis locus consists of two nonidentical genes. Comparative analysis of these genes revealed the presence of four conserved regions separated by three highly variable regions. B-cell epitope analysis identified three major cross-reacting epitopes that map to the variable regions. Location of the epitopes at the variable regions and the presence of multigene family with only one expressed copy suggest a mechanism of immune evasion in these Ehrlichiae.

L20 ANSWER 26 OF 29 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 1998084465 MEDLINE
DOCUMENT NUMBER: 98084465 PubMed ID: 9423849
TITLE: Immunodominant major outer membrane proteins of **Ehrlichia chaffeensis** are encoded by a polymorphic multigene family.
AUTHOR: Ohashi N; Zhi N; Zhang Y; Rikihisa Y
CORPORATE SOURCE: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus 43210-1093, USA.
CONTRACT NUMBER: RO1 AI33123 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1998 Jan) 66 (1) 132-9.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF021338; GENBANK-U72291
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980206
Last Updated on STN: 20000303
Entered Medline: 19980127

AB Several immunodominant major proteins ranging from 23 to 30 kDa were identified in the outer membrane fractions of **Ehrlichia chaffeensis** and **Ehrlichia** canis. The N-terminal amino acid sequence of a **28-kDa** protein of E. chaffeensis (one of the major proteins) was determined. The gene (**p28**), almost full length, encoding the **28-kDa** protein was cloned by PCR with primers designed based on the N-terminal sequence of the E. chaffeensis **28-kDa** protein and the consensus sequence between the C termini of the Cowdria ruminantium MAP-1 and Anaplasma marginale MSP-4 proteins. The **p28** gene was

overexpressed, and antibody to the recombinant protein was raised in a rabbit. The antibody and serum from a patient infected with E. chaffeensis reacted with the recombinant protein, three proteins (29, **28**, and 25 **kDa**) of E. chaffeensis, and a 30-kDa protein of E. canis. Immunoelectron microscopy with the rabbit antibody revealed that the antigenic epitope of the **28-kDa** protein was exposed on the surface of E. chaffeensis. Southern blot analysis with a 32P-labeled **p28** gene probe revealed multiple copies of genes homologous to **p28** in the E. chaffeensis genome. Six copies of the **p28** gene were cloned and sequenced from the genomic **DNA** by using the same probe. The open reading frames of these gene copies were tandemly arranged with intergenic spaces. They were nonidentical genes and contained a semivariable region and three hypervariable regions in the predicted protein molecules. One of the gene copies encoded a protein with an internal amino acid sequence identical to the chemically determined N-terminal amino acid sequence of a 23-kDa protein of E. chaffeensis. Immunization with the recombinant **P28** protein protected mice from infection with E. chaffeensis. These findings suggest that the 30-kDa-range proteins of E. chaffeensis represent a family of antigenically related homologous proteins encoded by a single gene family.

L20 ANSWER 27 OF 29 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 1998043955 MEDLINE
DOCUMENT NUMBER: 98043955 PubMed ID: 9384299
TITLE: Western immunoblotting analysis of the antibody responses of patients with human monocytotropic ehrlichiosis to different strains of **Ehrlichia chaffeensis** and **Ehrlichia** canis.
AUTHOR: Chen S M; Cullman L C; **Walker D H**
CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston 77555-0609, USA.
CONTRACT NUMBER: AI31431 (NIAID)
SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1997 Nov) 4 (6) 731-5.
Journal code: 9421292. ISSN: 1071-412X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980122
Last Updated on STN: 19980122
Entered Medline: 19980107

AB In order to evaluate the relative sensitivity of the detection of antibodies against various antigenic proteins of **Ehrlichia chaffeensis** for the diagnosis of the emerging infectious disease human monocytotropic ehrlichiosis, Western immunoblotting was performed with 27 serum samples from convalescent patients with antibodies, as demonstrated by indirect immunofluorescence assay. Among 22 patients with antibodies reactive with the 120-kDa protein, 15 showed reactivity with the 29/**28-kDa** protein(s) and the proteins in the 44- to 88-kDa range. Two of the serum samples with this pattern reacted with the 29/**28-kDa** protein(s) of only the 91HE17 strain, and one sample reacted with only that of the Arkansas strain, indicating that the antibodies were stimulated by strain-specific epitopes. Overall, antibodies to the 29/**28-kDa** protein(s) were detected in only 16 patients' sera, suggesting that this protein is less sensitive than the 120-kDa protein. Two of 12 serum samples from healthy blood donors had antibodies reactive with the 120-kDa protein; one of these

samples reacted also with the 29/**28-kDa** protein(s) of **Ehrlichia** canis, suggesting that unrecognized ehrlichial infection might have occurred, including human infection with E. canis. A high correlation between reactivity with the 120-kDa protein by Western immunoblotting and the recombinant 120-kDa protein by dot blot supports the potential usefulness of this recombinant antigen in diagnostic serology.

L20 ANSWER 28 OF 29 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 96208049 MEDLINE
DOCUMENT NUMBER: 96208049 PubMed ID: 8615456
TITLE: Analysis and ultrastructural localization of **Ehrlichia chaffeensis** proteins with monoclonal antibodies.
AUTHOR: Chen S M; Popov V L; Feng H M; **Walker D H**
CORPORATE SOURCE: Department of Pathology, University of Texas Medical Branch, Galveston, USA.
CONTRACT NUMBER: AI-314131 (NIAID)
SOURCE: AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, (1996 Apr) 54 (4) 405-12.
Journal code: 0370507. ISSN: 0002-9637.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960613
Last Updated on STN: 19960613
Entered Medline: 19960606

AB **Ehrlichia chaffeensis**, an obligately intracellular bacterium with tropism for monocytes, is the etiologic agent of human monocytic ehrlichiosis. To determine the nature and ultrastructural location of E. **chaffeensis** antigens, monoclonal antibodies (MAbs) to E. **chaffeensis** were developed. The MAbs were used for immunofluorescence and Western immunoblotting analysis of the antigens of density gradient-purified ehrlichiae. Monoclonal antibody 6A1 recognized an epitope of a 30-kD protein. This antibody reacted with a strain-specific epitope of E. **chaffeensis**, Arkansas strain, and did not cross-react with any other **ehrlichia** tested. Monoclonal antibodies 3C7 and 7C1-B recognized Arkansas strain proteins of 30 and 29 kD and reacted with E. **chaffeensis** (strain 91HE17) proteins of 31 and 29 kD and an E. canis protein of 30 kD. Lack of reactivity of these two MAbs with E. sennetsu and E. risticii suggests that the epitope is group-specific. Monoclonal antibody 5D11 recognized a 58-kD protein of both strains of E. **chaffeensis** as well as E. canis, apparently a group-specific, conformation-independent epitope. Monoclonal antibody 7C1-C reacted with 58- and 88-kD proteins of both the Arkansas and 91HE17 strains. Trypsin treatment destroyed the reactivity of E. **chaffeensis** antigens with all the MAbs when tested by Western immunoblotting, indicating that these antigens are proteins with trypsin-sensitive epitopes. Immunoelectron microscopy of negatively stained intact E. **chaffeensis** organisms showed that the 30- and 29-kD proteins are present on the surface of the ehrlichial cell wall along with the previously localized **28-kD** protein.

L20 ANSWER 29 OF 29 MEDLINE DUPLICATE 19
ACCESSION NUMBER: 92259582 MEDLINE
DOCUMENT NUMBER: 92259582 PubMed ID: 1583101
TITLE: Antigenic characterization of ehrlichiae: protein

immunoblotting of **Ehrlichia** canis, **Ehrlichia** sennetsu, and **Ehrlichia** risticii.

AUTHOR: Brouqui P; Dumler J S; Raoult D; **Walker D H**
CORPORATE SOURCE: Centre National de References des Rickettsioses, Centre Hospitalier Universitaire Timone, Marseille, France.

SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1992 May) 30 (5) 1062-6.
Journal code: 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920626
Last Updated on STN: 19920626
Entered Medline: 19920617

AB In recent years a febrile illness apparently associated with tick bite in patients in the United States has been attributed to infection by an **Ehrlichia** species. This implication is based on serologic responses to E. canis, morphologic demonstration of ehrlichiae in clinical materials, and a single isolate distinct from E. canis which was obtained from a human patient by the Centers for Disease Control. Little is known about the antigens of the ehrlichiae. This report expands the breadth of available knowledge concerning the antigenic components and serologic responses to component antigens of E. canis, E. sennetsu, and E. risticii. Protein immunoblotting after sodium dodecyl sulfate-polyacrylamide gel electrophoresis by using density gradient-purified ehrlichiae and homologous antisera demonstrated reproducible and characteristic antigens within each species (for E. sennetsu, 91, 64, 54, 44, 36, 34, **28**, 25, and 24 **kDa**; for E. risticii, 70, 52, 48, 44, 35, 28, 24, 23, and 20 kDa; for E. canis, 110, 64, 52, 42, 33, 28, 24, 23, and 20 kDa). When antisera were reacted with heterologous antigens, cross-reactivity among these species was virtually restricted to the 70-kDa antigen. Furthermore, when serum samples obtained from 10 patients who were convalescing from ehrlichiosis were tested against each antigen, only three serum samples had any reactivities, and these serum samples reacted with only a few of the antigenic bands. These results documented the molecular sizes of electrophoretically separated antigens of the three **Ehrlichia** species, confirm their serologic relationships, and support the novel nature of the agent(s) of human ehrlichiosis in the United States.